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14. ABSTRACT

BRCA1 is implicated in DNA maintenance and repair, as well as in cell cycle control. We hypothesize that activation of BRCA1 in prostate tumors is a consequence of an increased proliferation rate as a compensatory mechanism of tumor cells to allow DNA repair in highly replicating cells. As such, tumor expression of BRCA1 is a marker of lethal prostate cancer. In the current study, we aim to confirm our results that BRCA1 positive tumors are characterized by a rapidly lethal phenotype and will correlate BRCA1 status in tumors with expression of cell cycle regulators, p27 and p21 in a large cohort of men with prostate cancer from the Health Professionals Follow-up Study. We will generate data on *BRCA1* mutations and germline polymorphisms to determine if they are associated with lethal prostate cancer, increased cellular proliferation, and expression of p27, p21, and BRCA1. This project's goals are to identify biomarkers of progression by examining BRCA1 tumor status and cancer mortality, as well as to understand aspects of tumor biology associated with BRCA1.

15. SUBJECT TERMS
Prostate cancer, lethal, BRCA1, cell cycle

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INTRODUCTION

BRCA1 (breast cancer 1, early onset) is a multifunctional gene implicated in various processes of DNA maintenance and repair.[1-3] Moreover, *BRCA1* is proposed in cell cycle control, whereby *BRCA1* induced arrest at different check-points of the cell-cycle to allow the necessary time required for DNA repair.[4-6] Few studies to date have examined prostate tumor expression of BRCA1 as a biomarker of lethal disease. Inherited mutations in *BRCA1*, usually resulting in non-functional, truncated proteins, have been implicated in substantially increased risk in breast and ovarian cancer.[7-9] Accumulating evidence also points to *BRCA1* germline mutations causing increased prostate cancer risk in specific study populations.[10, 11] While the associations of germ-line mutations in *BRCA1* on prostate cancer susceptibility have been examined in some detail, no study to our knowledge has examined germline mutations or common polymorphisms in relation to lethal prostate cancer, nor whether germline variation in *BRCA1* could influence protein tumor expression. Our hypothesis is that activation of *BRCA1* in a subset of prostate tumors is a direct consequence of the increased proliferation rate. This activation is a compensatory mechanism of the tumor cells in the attempt to allow DNA repair in rapidly replicating cells. If confirmed, our preliminary findings suggest *BRCA1* protein expression could be an important biomarker of lethal prostate cancer and help guide therapeutic decisions. In addition, these results will help advance our understanding the role of *BRCA1* in cell cycle control, and perhaps lead to identification of potential targets for new anti-cancer agents. The objective of the proposed study is to characterize the role of *BRCA1*, both at the tumor and DNA level, in prostate cancer progression. Specifically, we hypothesize that prostate tumors positive for *BRCA1* are characterized by a rapidly lethal phenotype, and that this association is due to a “last chance” compensatory mechanism by cells to overcome the highly proliferative nature of a subset of tumors. Moreover, we hypothesize that prostate cancer cases with *BRCA1* mutations or specific genetic variants are at increased risk of lethal prostate cancer, and that part of the association may be acting through regulation of *BRCA1* expression in tumors.

BODY

Specific Aim 1: BRCA1 tumor expression and lethal prostate cancer

Our approach to characterize *BRCA1* tumor status is to evaluate immunohistochemical expression of the *BRCA1* protein of the tumor tissue microarrays. Moreover, we propose to characterize the extent of tumor proliferation and two markers of cell cycle progression (p21 and p27).

During Year 1, we aimed to complete construction of the tumor tissue microarrays and this is indeed complete. Our pathology team has now completed the histopathological review of the cases for the identification of tumor tissue, as well as to provide a standardized review for Gleason grading and other histological features. We have constructed three additional tumor microarrays for a total of 8 tumor TMAs for the Health Professionals Follow-up Study (HPFS) and 6 tumor TMAs for the Physicians' Health Study (PHS). Each of the prostate cancer cases is included as 3 replicate cores per case. Task 1 is now complete.

Task 2 was the measurement of the tumor biomarkers on the tissue microarrays, and we have initiated immunohistochemical evaluation of the proposed tumor markers. We have optimized the staining protocol for *BRCA1* and ki67 immunohistochemistry (as shown in **Supporting Data**, Figure 1). Staining is complete, and we will complete immunohistochemical evaluation and image analysis for these two markers during the next months. Moreover, we have begun optimizing the staining protocols for p21 and p27 on the HPFS test tumor microarrays and have excellent results. We will proceed with the staining of the test arrays during the next few months. We have achieved our major benchmarks for Task 2 during the first year of the grant.

An important achievement of this year has been the clinical review of the tumor cohort. The endpoints committee is up to date on the review of death certificates and medical records and assigning cause of death for the men in the cohort who have died through March 2011. For the current proposal, we will continue the follow-up through 2012, and the endpoints committee will continue to review for cause of death. Information on metastases and recurrence measures is also on track. We constructed an Access database and data entry form for the standardized extraction of medical record information for PSA measurements, additional treatments, development of metastases and other related clinical information. We have completed a detailed medical record review of 450 of the men in the cohort, and this work will continue into the second year of the project. Thus, we have made substantial progress on Task 3 of this aim.

We recently published our findings on *BRCA1* protein expression and lethal prostate cancer in the Physicians' Health Study in Cancer Research (See **Appendix 1**). In this study, we found that 15 percent of the prostate tumors stained positive for *BRCA1*. *BRCA1*-positive tumors had substantially increased tumor proliferation index compared with negative tumors (47.0 Ki67-positive nuclei versus 10.3, P = 0.0016) and were more likely to develop lethal cancer compared with BRCA1-negative tumors (hazard ratio, 4.6; 95% confidence interval, 2.4-8.7, **Supporting Data**, Figure 2). These findings strengthen the hypothesis that *BRCA1* plays a role in cell cycle control and show that *BRCA1* is a marker of clinical prostate cancer prognosis. These data were also presented as a poster presentation at the US Army Prostate Cancer Research Program ImPACT meeting in Orlando 2011 (See **Appendix 2**)

Specific Aim 2: Germline mutations and polymorphisms in BRCA1 and lethal prostate cancer

Much of the preliminary work summarized in Aim 1 is directly relevant to Aim 2 of the project, including the clinical data review, tissue retrieval, histologic evaluation, TMA construction, and immunohistochemical assessment. Below we summarize the progress on the additional tasks for this Aim.

In this aim, we seek to evaluate the extent to which germline mutations and common single nucleotide polymorphisms in *BRCA1* are linked with an increased risk of lethal prostate cancer. In Task 1, we worked towards the selection of variants in *BRCA1* for genotyping. We are now in the process of undertaking a haplotype tagging approach to characterize the genetic variation of common SNPs across *BRCA1*. Moreover, we are in the process of reviewing the literature to identify common germline mutations in *BRCA1* for genotyping. This work is ongoing and should be completed in the next two months.

Task 2 is focused on the DNA extraction from benign tissue from the men in the TMA cohort. We have cored benign tissue and extracted the DNA from the first 400 cases. Our strategy is to take 5 0.6 mm TMA cores of tissue and extract on the Qiagen BioRobot. With this approach, we are averaging 2000 ng total DNA per case. As part of this task, we have also determined the plating arrangement for the cases, including various quality control assessments. The remaining extractions will take place within the next 3 months, and then genotyping will begin.

In preliminary analyses, we used existing data from a genome wide association study among 630 prostate cancer cases in the Physicians' Health Study that were genotyped using Affymetrix. We studied six single nucleotide polymorphisms (SNPs) in *BRCA1* in relation to cancer-specific mortality, and found all six *BRCA1* SNPs were positively associated with lethal prostate cancer (Odds Ratios~1.29-1.34, p-trend~.02-.04). The SNPs from the Affymetrix platform are in such strong linkage disequilibrium that one SNP characterizes the other SNPs. This preliminary analysis provides compelling data to support the hypothesis, and we will extend the findings in a more comprehensive analysis within the current project.

KEY RESEARCH ACCOMPLISHMENTS

- Completed pathologic review of cohort on Gleason grading and constructed new tumor tissue microarrays
- Completed biomarker studies on tumor tissue microarrays to assess protein expression of *BRCA1* and cellular proliferation
- Created an Access database for standardized collection of clinical database. This resource will be used for future projects
- Completed review of tissue specimens for identifying histologically normal tissue and completed DNA extraction with excellent DNA yields
- Published manuscript on BRCA1 protein expression of lethal prostate cancer in *Cancer Research* and presented results at the US Army Prostate Cancer ImPACT meeting in 2011
- Undertook analyses using existing GWAS data and identified SNPs in *BRCA1* that are associated with risk of lethal prostate cancer

REPORTABLE OUTCOMES

- New student working on this project (Irene Shui) defended her thesis and was appointed as Post-doctoral fellow at the Harvard School of Public Health
- Research Fellow working on the project (Kathryn Penney) is now being appointed as Instructor of Medicine at Harvard Medical School
- Dr. Mucci was named the Outstanding Young Investigator from the Prostate Cancer Foundation based on experience supported by this award
- Development of prostate tumor tissue microarrays of the HPFS cohort
- Presented results at national research meeting
    - US Army Prostate Cancer Program ImPACT Meeting, Orlando, FL
- Published manuscript
    - Fiorentino M, Judson G, Penney K, Flavin R, Stark J, Fiore C, Fall K, Martin N, Ma J, Sinnott J, Giovannucci E, Stampfer M, Sesso HD, Kantoff PW, Finn S, Loda M, Mucci L. Immunohistochemical expression of BRCA1 and lethal prostate cancer. Cancer Res. 2010; 70: 3136-9.

CONCLUSION

We have demonstrated our ability to undertake this large patho-epidemiology cohort and create a biorepository of tumor tissue microarrays from men in the Health Professionals Follow-up Study. We have demonstrated a proven working relationship with the pathology team, as shown by completion of the construction of the tissue microarrays, standardized Gleason grading, and successful completion of biomarkers on the tissue microarrays. Moreover, our statistical analyses on *BRCA1* and prostate cancer-specific mortality, as well as the SNP analysis in *BRCA1* provide supportive evidence for the study hypothesis that *BRCA1* plays a key role in the progression of prostate cancer.

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SUPPORTING DATA

Figure 1. Co-expression of protein tumor expression of BRCA1 and ki67 in prostate cancer, stratified by Gleason grade

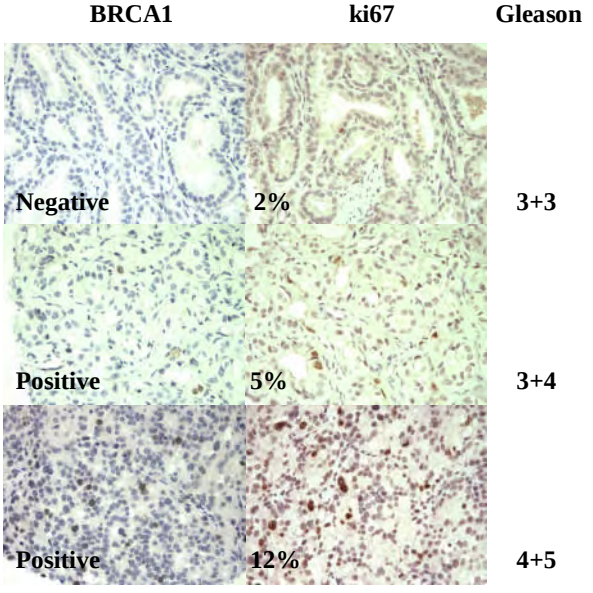
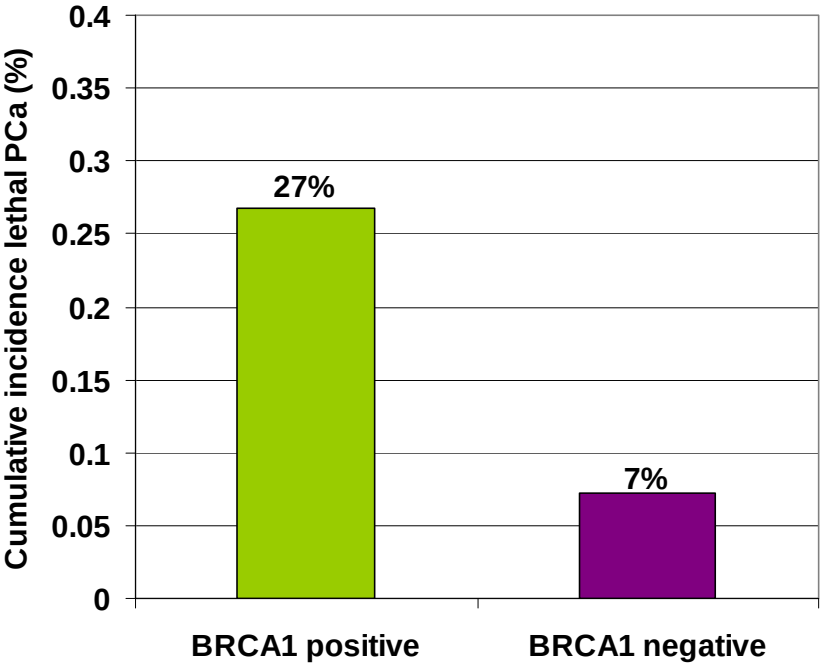


Figure 2. Cumulative incidence of lethal prostate cancer among BRCA1 positive and negative prostate cancer during a median 13 years of clinical follow-up.



LIST OF APPENDICES

1. Manuscript: Fiorentino M, Judson G, Penney K, Flavin R, Stark J, Fiore C, Fall K, Martin N, Ma J, Sinnott J, Giovannucci E, Stampfer M, Sesso HD, Kantoff PW, Finn S, Loda M, Mucci L. Immunohistochemical expression of BRCA1 and lethal prostate cancer. Cancer Res. 2010; 70(8): 3136-9.

2. Abstract: Tumor expression of BRCA1 and Lethal Prostate Cancer. Presented at the 2011 US Army Prostate Cancer Research Program, Orlando FL

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Immunohistochemical Expression of BRCA1 and Lethal Prostate Cancer

Michelangelo Fiorentino, Gregory Judson, Kathryn Penney, et al.

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Cancer Research

Immunohistochemical Expression of BRCA1 and Lethal Prostate Cancer

Michelangelo Fiorentino[1,9], Gregory Judson[2,3], Kathryn Penney[2,3], Richard Flavin[1], Jennifer Stark[2,3], Christopher Fiore[1], Katja Fall[2,3,10], Neil Martin[3,6], Jing Ma[2], Jennifer Sinnott[2,5], Edward Giovannucci[2,3,4], Meir Stampfer[2,3,4], Howard D. Sesso[3,7], Philip W. Kantoff[8,11], Stephen Finn[1], Massimo Loda[1,11], and Lorelei Mucci[2,3]

Abstract

BRCA1 functions as a tumor suppressor; recent work suggests that BRCA1 may also induce cell cycle arrest to allow for DNA repair. We hypothesized that BRCA1 expression in prostate tumor tissue may be associated with prostate cancer progression through regulation of the cell cycle. We used immunohistochemistry to evaluate BRCA1 protein expression in archival tumor samples from 393 prostate cancer cases in the Physicians' Health Study. The men were followed prospectively from diagnosis to development of metastases and mortality. Fifteen percent of tumors stained positive for BRCA1. BRCA1-positive tumors had substantially increased tumor proliferation index compared with negative tumors (47.0 Ki67-positive nuclei versus 10.3, $P = 0.0016$) and were more likely to develop lethal cancer compared with BRCA1-negative tumors (hazard ratio, 4.6; 95% confidence interval, 2.4–8.7). These findings strengthen the hypothesis that BRCA1 plays a role in cell cycle control and show that BRCA1 is a marker of clinical prostate cancer prognosis. *Cancer Res; 70(8); 3136–9. ©2010 AACR.*

Introduction

BRCA1 is a multifunctional tumor suppressor protein implicated in regulating the maintenance of genome integrity through the activation of DNA repair genes, heterochromatin formation, double strand–break repair, homologous recombination events, and ubiquitination (1–3). Recently, a more complex role for *BRCA1* was proposed, whereby BRCA1 can induce arrest at different cell cycle check points to allow for DNA repair (4–6).

Mutations in *BRCA1* have been associated with increased risk of breast, ovarian, and, more recently, prostate cancer—particularly high-grade disease (7–12). However, although mutations in *BRCA1* may influence familial prostate cancer risk and progression, few studies have examined BRCA1 protein expression in prostate cancer tumor tissue, and, to our knowledge, none have correlated expression with cancer progression and mortality. Recently, Schayek and colleagues showed that BRCA1 protein expression in prostate differentially regulates *IGF-IR* gene expression in an androgen-dependent manner and found significantly elevated BRCA1 levels in prostate cancer in comparison with normal prostate tissue (13). We hypothesized that BRCA1 expression could have prognostic relevance in prostate cancer through its regulation of the cell cycle regardless of germ-line mutations.

Materials and Methods

We undertook a prospective study among 392 men in the Physicians' Health Study (refs. 14, 15; http://clinicaltrials.gov identifier: NCT00000500) who were diagnosed with prostate cancer from 1983 to 2004. We constructed tumor tissue microarrays from archival prostatectomy and trans urethral resection of the prostate tumor tissue specimens using three 0.6-mm cores of tumor per case. Immunohistochemical staining was performed on 5-μm sections of the tissue microarrays (TMA) to assess BRCA1 expression [monoclonal MS110 antibody specific for the NH_2 terminus of the 220 kDa BRCA1 protein (Calbiochem), diluted 1:50 after EDTA-based antigen retrieval] and cell proliferation [polyclonal anti Ki67 antibody (Vector Labs), diluted 1:2,000 after citrate-based antigen retrieval]. MCF7 and HCC1937 cell lines were used as positive and negative controls for BRCA1 immunostaining, respectively. Because of the small proportion of stained nuclei and the homogeneous intensity of the immunostaining, the study pathologists (M.F. and R.F.) scored tumor expression of BRCA1 manually as positive or negative; Ki67 proliferation index was scored by quantitative image analysis (Ariol SL-50,

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Note: M. Loda and L. Mucci share senior authorship.

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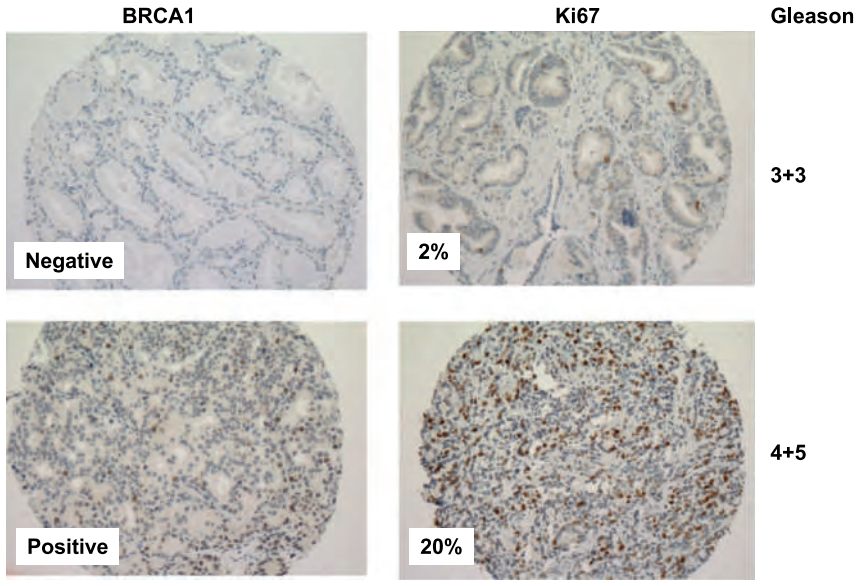

Figure 1. Comparative representation of BRCA1 expression and Ki67 proliferative index in serial sections of prostate cancer tissues with different Gleason scores. The brown immunostaining is nuclear for both BRCA1 and Ki67; slides are counterstained with hematoxylin (magnification ×200).

Applied Imaging; Fig. 1). The possible heterogeneity of the immunohistochemical staining for BRCA1 was also controlled using whole sections of 14 prostate cancer cases included in the TMAs. RNA expression levels of BRCA1 were available from a subset of participants ($n = 116$) using a gene expression profiling study that applied the DASL Illumina 6K array (16). The study was approved by Partners Health Care Institutional Review Board.

We abstracted data on age, stage, and prostate specific antigen (PSA) levels at diagnosis from medical records, and conducted a standardized histopathologic review for Gleason score (17, 18). The men were followed prospectively since diagnosis for the development of bony metastases and mortality through March 2009, without loss to follow-up.

We evaluated whether BRCA1-positive and BRCA1-negative tumor status based on immunohistochemistry differed according to Gleason score, tumor stage, PSA level, and age at diagnosis using generalized linear regression for continuous data and χ^2 tests for categorical data. In addition, we assessed BRCA1-positive and BRCA1-negative prostate tumors for the number of Ki67-positive nuclei as well as BRCA1 RNA expression levels using ANOVA. Mean Ki67-positive

Table 1. Clinical characteristics of 392 men in the Physicians' Health Study according to BRCA1 status, 1983 to 2008

	BRCA1 negative	BRCA1 positive	*P*
n	332	60	
Age at diagnosis (95% CI)	66.5 (65.7–67.2)	67.3 (65.6–69.0)	0.37
PSA at diagnosis (95% CI)	10.2 (6.0–14.4)	27.0 (15.9–38.1)	0.0056
Mean follow-up time	11.0 (10.5–11.4)	8.8 (7.7–9.9)	0.0006
n dead/metastases (% of total)	24 (7.2)	16 (26.7)	<0.0001
Gleason score, *n* (%)			0.004*
4–6	97 (29.2)	10 (16.7)	
3+4	116 (34.9)	19 (31.7)	
4+3	65 (19.6)	10 (16.7)	
8–10	52 (15.7)	21 (35.0)	
Stage, *n* (%)			0.0005*
pT_2	207 (62.3)	26 (43.3)	
pT_3	72 (21.7)	4 (6.7)	
pT_4/N_1	4 (1.2)	4 (6.7)	

*P for trend.

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nuclei scores were $\log_{10}$ transformed before analysis to account for the uneven distribution of scores in the raw data. To assess the extent to which BRCA1 status was associated with poor progression, we used Cox proportional hazards models and examined the association between BRCA1 status and lethal prostate cancer, defined as development of distant metastases or prostate cancer–specific mortality. All statistical tests were two-sided.

This project was approved by the Partners Health Care Institutional Review Board.

Results

Normal prostate tissue did not stain for BRCA1; however, 15.3% (n = 60) of prostate tumor samples showed patchy nuclear positive immunostaining with a punctuate pattern (Fig. 1). There was a total correspondence between BRCA1 staining in the TMA cores and in the whole sections obtained from the selected 14 corresponding donor blocks in terms of signal intensity and percentage of positive nuclei. Cases that stained positively for BRCA1 had substantially and significantly higher Gleason score, higher PSA levels at diagnosis, and more advanced stage compared to those with tumors that did not stain for BRCA1 (Table 1). Moreover, BRCA1-positive tumors were marked by substantially increased tumor proliferation index compared with BRCA1-negative tumors (47.0 Ki67-positive nuclei versus 10.3, P = 0.0016). Tumors staining positive for BRCA1 also showed increased BRCA1 mRNA relative expression [mean, 10.5; 95% confidence interval (95% CI), 10.2–10.8] compared with tumors negative for BRCA1 (mean, 9.9; 95% CI, 9.7-10.1, P for difference = 0.008).

During a mean follow-up of 10.6 years, 40 men died of cancer or developed bony metastases. Sixteen of the 60 men (26.7%) with BRCA1-positive tumors died of prostate cancer, compared with 24 of 332 (7.2%) men who were BRCA1 negative [hazard ratio (HR), 4.6; 95% CI, 2.4–8.7]. This association remained statistically significant after adjusting for age at diagnosis and Gleason score (HR, 2.5; 95% CI, 1.3–4.8). Interestingly, although BRCA1-positive tumors had substantially increased tumor proliferative index, the association of BRCA1 and lethal prostate cancer remained significant after controlling for $\log_{10}$-transformed Ki67 expression (HR, 3.6; 95% CI, 1.6–8.0).

Discussion

This study represents the first demonstration of a direct correlation between the expression of BRCA1 and the Ki67 proliferative index in prostate cancer and further strengthens the hypothesis that BRCA1 may play a role in cell cycle control and is a potent independent marker of clinical prognosis. Ki67 is a well-known predictor of adverse prognosis and resistance to therapy in prostate cancer (19, 20). In addition, association of increased proliferation and BRCA1 protein immunohistochemical expression was recently described in breast cancer epithelial cells from BRCA1 mutation carriers possibly as a result of epidermal growth factor receptor pathway activation (21). In agreement with the recent observation by Schayek and colleagues (13), we found that BRCA1 was not expressed in normal prostate tissue. We hypothesize that this localization of BRCA1 only to the most aggressive tumors may reflect an inefficient attempt to upregulate DNA repair mechanisms in prostate epithelial cells with high proliferative rate and extensive genetic instability.

Cases whose prostate tumors stained positive for BRCA1 had significantly higher Gleason score, PSA at diagnosis, and tumor proliferation as well as significantly worse prognosis than those with negative BRCA1 staining. In addition, mRNA levels were also increased in the BRCA1 protein–positive tumors, indicating a transcriptional-level control in these cases. Taken together, these observations support the hypothesis that the BRCA1 gene may hold another biological function apart from its tumor suppressor activity.

Although the mechanism of cell cycle regulation by BRCA1 still requires further exploration, we can conclude that the immunohistochemical investigation of BRCA1 protein expression represents a new tool for understanding the cell cycle regulation in the development of prostate cancer to lethal disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Tumor Expression of BRCA1 and Lethal Prostate Cancer

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BACKGROUND

BRCA1

BRCA1 acts as a tumor suppressor with a role in maintenance of genome integrity and DNA repair

A more complex role for was proposed as a cell-cycle regulator of transcription

BRCA1 and Prostate Cancer

Germ-line mutations of *BRCA1* confer susceptibility to develop prostate cancer in males

Prostate cancers arising in *BRCA1* mutation carriers have higher Gleason score with potential for more aggressive clinical behavior

Studies on the role of BRCA1 protein expression in prostate tumor tissue have reported:

- Elevated BRCA1 levels in prostate cancer in comparison with normal prostate tissue
- BRCA1 protein expression in prostate differentially regulates *IGF-IR* gene expression in an androgen-dependent manner

→No study to date has examined clinical outcomes related to BRCA1 protein expression

HYPOTHESIS

BRCA1 expression in prostate tumors has prognostic relevance in prostate cancer through its regulation of the cell-cycle

METHODS

Physicians' Health Study (PHS) Prostate Cancer Cohort, 1982-2008

- Men with prostate cancer (diagnosed 1982-2004) who are participants of PHS
- Archival tumor tissue available for N=392 men included on 4 tumor tissue microarrays
- Follow-up for bone metastases and cancer-specific mortality cancer through May 2008

Tumor Biomarkers

- Immunostained for BRCA1 (monoclonal MS110 antibody specific for the N-terminus Calbiochem), diluted 1:50 after EDTA-based antigen retrieval
- Study pathologists manually scored tumor expression of BRCA1, given small proportion of stained nuclei and homogeneous intensity of immunostaining
- Cell proliferation assessed (polyclonal anti Ki67 antibody, Vectorlab), diluted 1:2000 after citrate-based antigen retrieval
- RNA expression levels of BRCA1 available from subset (n=116) using a gene expression profiling study that applied the DASL Illumina 6K array

Statistical methods

- Cox regression models to study association of BRCA1 expression and lethal prostate cancer, with follow-up from diagnosis through May 2008 (mean 10.6 years)

RESULTS

Summary of key findings

- In the PHS, 60 of 392 (15.3%) prostate cancers showed positive nuclear (>5% tumor cells) staining for BRCA1 (Table 1)
- Normal prostate tissue adjacent to tumors always stained negative for BRCA1
- BRCA1 protein staining correlated strongly with mRNA expression levels suggesting transcriptional level control (BRCA1 positive Mean, 95% CI : 10.5, 10.2-10.8 vs. BRCA1 negative 9.9, 9.7-10.1, p for difference=0.008)
- BRCA1 positive cases were characterized by higher Gleason score, more advanced stage, and greater PSA levels at diagnosis (Table 1)
- BRCA1 positive tumors had four times greater Ki67 index compared to BRCA1 negative tumors (P for difference <.001) (Figure 1, 2)
- Men with BRCA1 positive tumors were 4 times more likely to develop lethal prostate cancer compared to negative tumors during follow-up (HR=4.6, 95% CI=2.4-8.7) (Figure 3)
- Association with lethal disease remained significant even controlling for Gleason and ki67 (HR=3.6, 95% CI=1.6-8.0)

Table 1. Clinical characteristics of prostate cancer cases in Physicians' Health Study, overall and by BRCA1 tumor status, 1982-2008

	All cases	BRCA+	BRCA-
Total, N	**393**	**60**	**332**
Lethal PCa, N (%)	**40**	**16 (26.7%)**	**24 (7.2%)**
Mean PSA at dx, ng/ml	**13.0 ng/ml**	**27.0 ng/ml**	**10.2 ng/ml**
Age at diagnosis, years	**67 yrs**	**67 yrs**	**66 yrs**
Gleason grade, %			
2-6	**27%**	**17%**	**29%**
3+4	**34%**	**32%**	**35%**
4+3	**19%**	**62%**	**20%**
8-10	**19%**	**35%**	**16%**
Pathologic stage, %			
T2	**75%**	**76%**	**74%**
T3	**21%**	**12%**	**23%**
T4/N1	**4%**	**12%**	**1%**

Figure 1. Co-expression of BRCA1 protein expression and ki67 staining by Gleason grade, PHS

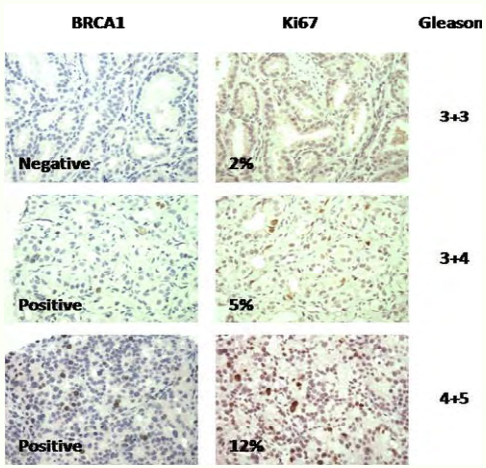


Figure 2. BRCA1 positive tumors have increased rates of cell proliferation compared to negative tumors, PHS

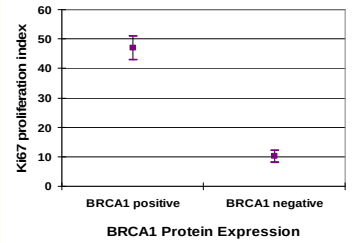
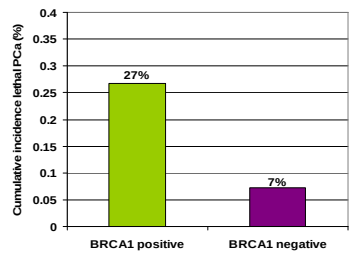


Figure 3. Cumulative incidence of lethal prostate cancer among BRCA1 positive and negative tumors



CONCLUSIONS

- BRCA1 is highly expressed in a subset of prostate tumors, but never in adjacent normal
- BRCA1 positive tumors are characterized by an aggressive phenotype, notably with a strong link with lethal disease
- BRCA1 positive tumors are also characterized by substantial increased rates of cell proliferation
- BRCA1 protein expression in prostate tumors supports its role as a regulator of cell-cycle transcription
- Localization of BRCA1 in the most aggressive tumors may reflect an inefficient attempt to regulate DNA repair in prostate epithelium undergoing rapid proliferation and genetic instability

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